

Lit Lunch 5/16/14

KEITH:

1) Hierarchical Functional Specificity of Cytosolic Heat Shock Protein 70 (Hsp70) Nucleotide Exchange Factors in Yeast

May 9, 2014 *The Journal of Biological Chemistry*, 289, 13155-13167.

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Heat shock protein 70 (Hsp70) molecular chaperones play critical roles in protein homeostasis. In the budding yeast *Saccharomyces cerevisiae*, cytosolic Hsp70 interacts with up to three types of nucleotide exchange factors (NEFs) homologous to human counterparts: Sse1/Sse2 (Heat shock protein 110 (Hsp110)), Fes1 (HspBP1), and Snl1 (Bag-1). All three NEFs stimulate ADP release; however, it is unclear why multiple distinct families have been maintained throughout eukaryotic evolution. In this study we investigate NEF roles in Hsp70 cell biology using an isogenic combinatorial collection of NEF deletion mutants. Utilizing well-characterized model substrates, we find that Sse1 participates in most Hsp70-mediated processes and is of particular importance in protein biogenesis and degradation, whereas Fes1 contributes to a minimal extent. Surprisingly, disaggregation and resolubilization of thermally denatured firefly luciferase occurred independently of NEF activity. Simultaneous deletion of SSE1 and FES1 resulted in constitutive activation of heat shock protein expression mediated by the transcription factor Hsf1, suggesting that these two factors are important for modulating stress response. Fes1 was found to interact *in vivo* preferentially with the Ssa family of cytosolic Hsp70 and not the co-translational Ssb homolog, consistent with the lack of cold sensitivity and protein biogenesis phenotypes for *fes1*Δ cells. No significant consequence could be attributed to deletion of the minor Hsp110 SSE2 or the Bag homolog SNL1. Together, these lines of investigation provide a comparative analysis of NEF function in yeast that implies Hsp110 is the principal NEF for cytosolic Hsp70, making it an ideal candidate for therapeutic intervention in human protein folding disorders.

2) A New Role for *Escherichia coli* DsbC Protein in Protection against Oxidative Stress

May 2, 2014 The Journal of Biological Chemistry, 289, 12356-12364.

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We report a new function for *Escherichia coli* DsbC, a protein best known for disulfide bond isomerization in the periplasm. We found that DsbC regulates the redox state of the single cysteine of the L-arabinose-binding protein AraF. This cysteine, which can be oxidized to a sulfenic acid, mediates the formation of a disulfide-linked homodimer under oxidative stress conditions, preventing L-arabinose binding. DsbC, unlike the homologous protein DsbG, reduces the intermolecular disulfide, restoring AraF binding properties. Thus, our results reveal a new link between oxidative protein folding and the defense mechanisms against oxidative stress.

DAMIAN:

1) Shuanglong Huang, Robert D. Hill, Owen S.D. Wally, Giuseppe Dionisio, Belay T. Ayele, Sravan Kumar Jami, Claudio Stasolla

Hemoglobin control of cell survival/death decision regulates *in vitro* plant embryogenesis

Plant Physiology Preview (May 2014)

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2) Lida Katsimpardi, Nadia K. Litterman, Pamela A. Schein, Christine M. Miller, Francesco S. Loffredo, Gregory R. Wojtkiewicz, John W. Chen, Richard T. Lee, Amy J. Wagers, Lee L. Rubin

Vascular and Neurogenic Rejuvenation of the Aging Mouse Brain by Young Systemic Factors

Science 344: 630 (9 May 2014)

Age-related cognitive decline is accompanied by a number of neurobiological corollaries, including decreased stem cell niches and reduced cerebral blood flow. These authors found that by either performing parabioses between old and young mice, or by exposing old mice to the TGF-beta family protein GDF11 (whose role in cardiac muscle regeneration was recently shown), old mice brain physiology changed to partially resemble that of younger mice. Specifically, neural stem cell numbers increased, cerebral blood flow was higher and brain blood vessel volumes were larger and more branched. Heterochronic old mice were also more sensitive to olfactory cues than were isochronic controls, in line with the observation that the olfactory bulbs were found to exhibit a significant amount of neurogenesis.

3) Mengmeng Zhu¹, Ning Zhu, Wen-yuan Song, Alice C. Harmon, Sarah M. Assmann, and Sixue Chen

Thiol-based redox proteins in abscisic acid and methyl jasmonate signaling in Brassica napus guard cells

The Plant Journal (2014) 78, 491–515

The authors were looking for guard cell proteins whose cysteine oxidation state was influenced by the hormones ABA and MeJA. ICAT (isotope-coded affinity tagging) and DIGE (differential in-gel electrophoresis) approaches were implemented using two different isotope tags and two different fluorors, respectively. The authors argue, unconvincingly at least to me, that ICAT is more capable of discerning differences due to protein abundance change than is DIGE. At any rate, cysteine modification within particular peptides was found to be sensitive to these two phytohormones for a variety of proteins, although the fold-change was usually only 1.5-2-fold different. Validation was carried out for two of the identified targets: A SnRK2 and an isopropyl-malate dehydrogenase. Indeed, oxidants such as GSNO and H₂O₂ and the reducing agent DTT influenced opposing trends in SnRK2 autophosphorylation and IPM dehydrogenase activity. Overall, this investigation yielded a putative “catalog” of ABA/MeJA redox switch proteins in guard cells, but I’m not clear on how they can distinguish changes in cysteine modification from changes in protein abundance.

NATHEN:

1) Active cage mechanism of chaperonin-assisted protein folding demonstrated at single molecule level

Gupta, Amit J.
Halder, Shubhasis
Miličić, Goran
Hartl, F. Ulrich
Hayer-Hartl, Manajit

Journal of Molecular Biology

The cylindrical chaperonin GroEL and its lid-shaped cofactor GroES of E. coli have an essential role in assisting protein folding by transiently encapsulating non-native substrate in an ATP-regulated mechanism. It remains controversial, whether the chaperonin system functions solely as an infinite dilution chamber, preventing off-pathway aggregation, or actively enhances folding kinetics by modulating the folding energy landscape. Here we developed single molecule approaches to distinguish between passive and active chaperonin mechanisms. Using low protein concentrations (100 pM) to exclude aggregation, we measured the spontaneous and GroEL/ES-assisted folding of double mutant maltose binding protein (DM-MBP) by single-pair fluorescence resonance energy transfer and fluorescence correlation spectroscopy. We find that GroEL/ES accelerates DM-MBP folding up to 8-fold over the spontaneous folding rate. Accelerated folding is achieved by encapsulation of folding intermediate in the GroEL/ES-cage, independent of repetitive cycles of protein binding and release from GroEL. Moreover, photoinduced electron transfer experiments provided direct physical evidence that the confining environment of the chaperonin restricts polypeptide chain dynamics. This effect is mediated by the net-negatively charged wall of the GroEL/ES cavity, as shown using the GroEL mutant EL(KKK2) in which the net-negative charge is removed. EL(KKK2)/ES functions as a passive cage in which folding occurs at the slow spontaneous rate. Taken together our findings suggest that protein encapsulation can accelerate folding by entropically destabilizing folding intermediates, in strong support of an active chaperonin mechanism in the folding of some proteins. Accelerated folding is biologically significant as it adjusts folding rates relative to the speed of protein synthesis.

2) HSP70 Transgene Directed Motion to Nuclear Speckles Facilitates Heat Shock Activation

- [Nimish Khanna¹](#), [Yan Hu^{1,2}](#), [Andrew S. Belmont¹](#)

Current Biology

Association and disassociation of gene loci with respect to specific nuclear compartments accompany changes in gene expression, yet little is known concerning the mechanisms by which this occurs or its functional consequences. Previously, we showed that tethering acidic activators to a peripheral chromosome site led to movement of the chromosome site away from the nuclear periphery, but the physiological relevance

of this movement was unclear [1]. Nuclear speckles, or interchromatin granule clusters, are enriched in factors involved in RNA processing [2], and the association of a subset of active genes at their periphery suggests speckles may play a role in gene expression [3 and 4]. Here, we show an actin-dependent association of HSP70 transgenes with nuclear speckles after heat shock. We visualized HSP70 transgenes moving curvilinearly toward nuclear speckles over ~0.5–6 μm distances at velocities of 1–2 μm min⁻¹. Chromatin stretching in the direction of movement demonstrates a force-generating mechanism. Transcription in nearly all cases increased noticeably only after initial contact with a nuclear speckle. Moreover, blocking new HSP70 transgene/speckle association by actin depolymerization prevented significant heat shock-induced transcriptional activation in transgenes not associated with speckles, although robust transcriptional activation was observed for HSP70 transgenes associated with nuclear speckles. Our results demonstrate the existence of a still-to-be-revealed machinery for moving chromatin in a direct path over long distances toward nuclear speckles in response to transcriptional activation; moreover, this speckle association enhances the heat shock activation of these HSP70 transgenes.

ANGELA:

1) Molecular Plant

A Special Issue on Plant Stress Biology: From Model Species to Crops

Wei Li and Xiaofeng Cui

Mol. Plant (2014) 7 (5): 755-757. doi: 10.1093/mp/ssu040

This special issue, organized by Drs Hans Bohnert, Ray Bressan, and Jian-Kang Zhu, collected one research highlight, three reviews, and 13 research articles. The research highlight by [de Zelicourt et al. \(2013\)](#) conducted an up-to-date analysis of the known roles of rhizosphere microbes, with a focus on the non-symbiotic bacteria and fungi in plant stress tolerance, and highlighted the usefulness of beneficial microbes in improving plant stress tolerance ([de Zelicourt et al., 2013](#)). Pamela Ronald and colleagues (2013) reviewed the recent advances in dissecting the crosstalk mechanisms between biotic and abiotic stress-responsive signaling pathways in rice. By highlighting the systems biology approaches for analyzing stress-regulatory networks, they discussed the crosstalk of signaling pathways mediated by three important regulators: XA21 which encodes a receptor kinase conferring resistance to *Xanthomonas oryzae* pv. *oryzae*, NH1 which is a key regulator of systemic acquired resistance, and SUB1A encoding an ethylene-responsive transcription factor crucial for tolerance to submergence stress ([Sharma et al., 2013](#)). As plant-specific transcription factors, members of the WRKY gene family play diverse roles in plant growth and development, and especially are critical for plant

responses to biotic and abiotic stresses. The review by Chi et al. (2013) summarized the recent findings of protein–protein interaction studies on WRKY transcription factors and discussed the regulatory roles and action mode of the interacting partners in the WRKY signaling networks (Chi et al., 2013).

2) Role of *Arabidopsis UV RESISTANCE LOCUS 8* in Plant Growth Reduction under Osmotic Stress and Low Levels of UV-B

Rossella Fasano^a, Nathalie Gonzalez^{b,c}, Alessandra Tosco^a, Fabrizio Dal Piaz^a, Teresa Docimo^d, Ramon Serrano^e, Stefania Grillo^f, Antonella Leone^a and Dirk Inzé^{b,c,1}

Mol. Plant (2014) 7 (5): 773-791. doi: 10.1093/mp/ssu002

In high-light environments, plants are exposed to different types of stresses, such as an excess of UV-B, but also drought stress which triggers a common morphogenic adaptive response resulting in a general reduction of plant growth. Here, we report that the *Arabidopsis thaliana* UV RESISTANCE LOCUS 8 (UVR8) gene, a known regulator of the UV-B morphogenic response, was able to complement a *Saccharomyces cerevisiae* osmo-sensitive mutant and its expression was induced after osmotic or salt stress in *Arabidopsis* plants. Under low levels of UV-B, plants overexpressing UVR8 are dwarfed with a reduced root development and accumulate more flavonoids compared to control plants. The growth defects are mainly due to the inhibition of cell expansion. The growth inhibition triggered by UVR8 overexpression in plants under low levels of UV-B was exacerbated by mannitol-induced osmotic stress, but it was not significantly affected by ionic stress. In contrast, *uvr8-6* mutant plants do not differ from wild-type plants under standard conditions, but they show an increased shoot growth under high-salt stress. Our data suggest that UVR8-mediated accumulation of flavonoid and possibly changes in auxin homeostasis are the underlying mechanism of the observed growth phenotypes and that UVR8 might have an important role for integrating plant growth and stress signals.

3) Analysis of Short-Term Metabolic Alterations in *Arabidopsis* Following Changes in the Prevailing Environmental Conditions

Alexandra Florian, Zoran Nikoloski, Ronan Sulpice, Stefan Timm, Wagner L. Araújo, Takayuki Tohge, Hermann Bauwe, and Alisdair R. Fernie

Mol. Plant (2014) 7 (5): 893-911 doi:10.1093/mp/ssu008

Although photorespiration is known to be regulated in response to temperature and light conditions, comprehensive systems-level information concerning such responses has not yet been recorded. For this reason, we here describe metabolic, transcriptional, protein, and physiological response to either independently increasing or decreasing light or temperature conditions from the growth conditions of *Arabidopsis*. The collated data suggest that the response to both treatments is similar and that it is not mediated at the level of transcription but rather by the inherent metabolic capacities of the leaf.

ELIZABETH:
May 5, 2014

Wang X, Chen M, Zhou J, Zhang X.
HSP27, 70 and 90, anti-apoptotic proteins, in clinical cancer therapy
(Review).
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PMID: 24789222 [PubMed - as supplied by publisher]

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Expression of small heat shock protein (sHSP) genes in the garden pea
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Understanding the Influence of Codon Translation Rates on
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Acc Chem Res. 2014 May 1;. [Epub ahead of print]
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Role of calmodulin in thermotolerance.
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PMID: 24781358 [PubMed - as supplied by publisher]

Zhong B, Lama R, Kulman DG, Li B, Su B.
Lead optimization of dual tubulin and Hsp27 inhibitors.
Eur J Med Chem. 2014 Apr 13;80C:243-253. [Epub ahead of print]
PMID: 24780601 [PubMed - as supplied by publisher]

Holmes WM, Klaips CL, Serio TR.
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PMID: 24569876 [PubMed - as supplied by publisher]
Huang S, Hill RD, Wally OS, Dionisio G, Ayele BT, Jami SK, Stasolla C.

Hemoglobin control of cell survival/death decision regulates in vitro
plant embryogenesis.
Plant Physiol. 2014 May 1;. [Epub ahead of print]
PMID: 24784758 [PubMed - as supplied by publisher]

STEPHANIE:

1) [Plant Cell Physiol](#). 2014 Apr;55(4):801-10. doi: 10.1093/pcc/pcu018. Epub 2014 Jan 30.

Multiple BiP Genes of *Arabidopsis thaliana* are Required for Male Gametogenesis and Pollen Competitiveness.

[Maruyama D](#)¹, [Sugiyama T](#), [Endo T](#), [Nishikawa S](#).

Author information

Abstract

Immunoglobulin-binding protein (BiP) is a molecular chaperone of the heat shock protein 70 (Hsp70) family. BiP is localized in the endoplasmic reticulum (ER) and plays key roles in protein translocation, protein folding and quality control in the ER. The genomes of flowering plants contain multiple BiP genes. *Arabidopsis thaliana* has three BiP genes. BIP1 and BIP2 are ubiquitously expressed. BIP3 encodes a less well conserved BiP paralog, and it is expressed only under ER stress conditions in the majority of organs. Here, we report that all BiP genes are expressed and functional in pollen and pollen tubes. Although the *bip1 bip2* double mutation does not affect pollen viability, the *bip1 bip2 bip3* triple mutation is lethal in pollen. This result indicates that lethality of the *bip1 bip2* double mutation is rescued by BiP3 expression. A decrease in the copy number of the ubiquitously expressed BiP genes correlates well with a decrease in pollen tube growth, which leads to reduced fitness of mutant pollen during fertilization. Because an increased protein secretion activity is expected to increase the protein folding demand in the ER, the multiple BiP genes probably cooperate with each other to ensure ER homeostasis in cells with active secretion such as rapidly growing pollen tubes.

KEYWORDS:

Arabidopsis thaliana, Endoplasmic reticulum, Male gametogenesis, Molecular chaperone, Pollen tube

INDU:

1. Science. 2014 Mar 28;343(6178):1509-12. doi: 10.1126/science.1248849. Epub 2014 Mar 13.

A bacterial tyrosine phosphatase inhibits plant pattern recognition receptor activation.

Macho AP(1), Schwessinger B, Ntoukakis V, Brutus A, Segonzac C, Roy S, Kadota Y, Oh MH, Sklenar J, Derbyshire P, Lozano-Durán R, Malinovsky FG, Monaghan J, Menke FL, Huber SC, He SY, Zipfel C.

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Innate immunity relies on the perception of pathogen-associated molecular patterns (PAMPs) by pattern-recognition receptors (PRRs) located on the host cell's surface. Many plant PRRs are kinases. Here, we report that the *Arabidopsis* receptor kinase EF-TU RECEPTOR (EFR), which perceives the elf18 peptide derived from bacterial elongation factor Tu, is activated upon ligand binding by phosphorylation on its tyrosine residues. Phosphorylation of a single tyrosine residue, Y836, is required for activation of EFR and downstream immunity to the

phytopathogenic bacterium *Pseudomonas syringae*. A tyrosine phosphatase, HopAO1, secreted by *P. syringae*, reduces EFR phosphorylation and prevents subsequent immune responses. Thus, host and pathogen compete to take control of PRR tyrosine phosphorylation used to initiate antibacterial immunity.

PMID: 24625928 [PubMed - indexed for MEDLINE]

2. *Science*. 2014 Apr 4;344(6179):55-8. doi: 10.1126/science.1249252. Epub 2014 Mar 27.

Total synthesis of a functional designer eukaryotic chromosome.

Annaluru N(1), Muller H, Mitchell LA, Ramalingam S, Stracquadanio G, Richardson SM, Dymond JS, Kuang Z, Scheifele LZ, Cooper EM, Cai Y, Zeller K, Agmon N, Han JS, Hadjithomas M, Tullman J, Caravelli K, Cirelli K, Guo Z, London V, Yeluru A, Murugan S, Kandavelou K, Agier N, Fischer G, Yang K, Martin JA, Bilgel M, Bohutski P, Boulier KM, Capaldo BJ, Chang J, Charoen K, Choi WJ, Deng P, DiCarlo JE, Doong J, Dunn J, Feinberg JI, Fernandez C, Floria CE, Gladowski D, Hadidi P, Ishizuka I, Jabbari J, Lau CY, Lee PA, Li S, Lin D, Linder ME, Ling J, Liu J, Liu J, London M, Ma H, Mao J, McDade JE, McMillan A, Moore AM, Oh WC, Ouyang Y, Patel R, Paul M, Paulsen LC, Qiu J, Rhee A, Rubashkin MG, Soh IY, Sotuyo NE, Srinivas V, Suarez A, Wong A, Wong R, Xie WR, Xu Y, Yu AT, Koszul R, Bader JS, Boeke JD, Chandrasegaran S.

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Comment in

Nature. 2014 May 8;509(7499):168-9.

Rapid advances in DNA synthesis techniques have made it possible to engineer viruses, biochemical pathways and assemble bacterial genomes. Here, we report the synthesis of a functional 272,871-base pair designer eukaryotic chromosome, synIII, which is based on the 316,617-base pair native *Saccharomyces cerevisiae* chromosome III. Changes to synIII include TAG/TAA stop-codon replacements, deletion of subtelomeric regions, introns, transfer RNAs, transposons, and silent mating loci as well as insertion of loxP sites to enable genome scrambling. SynIII is functional in *S. cerevisiae*. Scrambling of the chromosome in a heterozygous diploid reveals a large increase in a-mater derivatives resulting from loss of the MAT^α allele on synIII. The complete design and synthesis of synIII establishes *S. cerevisiae* as the basis for designer eukaryotic genome biology.

PMID: 24674868 [PubMed - indexed for MEDLINE]

ARIEL:

1) Small heat shock proteins and their role in meat tenderness: a review

Abstract: The eating quality of meat is a result of complex interactions between the biological traits and biochemical processes during the conversion of muscle to meat. It was hypothesised that muscles inevitably engage towards apoptotic cell death due to the termination of oxygen and nutrient supply to the muscle following exsanguination. Thus, factors that regulate the process of apoptotic cell death of muscle cells are believed to ultimately influence meat quality. Proteomic studies have associated the regulation of small heat shock proteins (sHSPs) with various meat quality attributes including tenderness, colour, juiciness and flavour. Due to the anti-apoptotic and chaperone functions of sHSPs, they are proposed to be involved with the eating quality of meat. In this review, we discuss the possible chaperone and anti-apoptotic role of sHSPs during the conversion of muscle to meat and consider the repercussions of this on the development of meat tenderness.